

REMARKS

Amendments

The specification has been amended to insert sequence identifiers. It is submitted that the insertion of sequence identifiers does not constitute new matter, and the entry of these amendments is requested.

Claim 1 has been amended to specify that the target sequence is located in the promoter of the gene of interest. Support for this language can be found in claim 2 as originally filed.

Claims 2, 3 and 5 have been canceled without prejudice for filing in one or more divisional or continuation applications.

Claim 22 has been amended to specify that the RASSF1 gene is human. Support for this amendment can be found at, for example, page 13, paragraph [00051].

It is submitted that these amendments do not constitute new matter, and their entry is requested.

A Sequence Listing has been provided. It is submitted that the Sequence Listing does not constitute new matter, and its entry is requested. A computer readable form of the Sequence Listing and an appropriate statement accompany this Amendment.

Summary of the Invention

The present invention is directed to method for directing DNA methylation in mammalian cells using homologous, short double stranded RNAs, also known as siRNAs. The method comprises exposing a mammalian cell to an siRNA molecule that is specific for a target sequence of the gene which is desired to be methylated. The target sequence is located in the promoter region of the gene of interest. Thus, the present invention provides for the DNA methylation of any region of a gene that can be methylated. The present invention has been illustrated by directing the methylation of the human RASSF1 gene, specifically the human RASSF1A gene, using siRNA. The human RASSF1 gene was known in the art at the time of the present invention, as evidenced by the

Examiner's citation of Dammann et al. (*Nature Genetics* **25**:315-319, 2000). The present invention is not directed to the RASSF1 gene *per se*.

Sequence Compliance

The Examiner noted that the application was not in compliance with the sequence requirements. Applicants have prepared and submit herewith a Sequence Listing, along with a computer readable form of the Sequence Listing and appropriate statement. It is believed that the submission of these items brings the application into compliance with the sequence requirements.

Written Description Rejection

The Examiner has rejected claim 22 under 35 U.S.C. § 112, first paragraph for lack of written description. The Examiner has rejected this claim for the use of the term RASSF1 gene without, in essence, any specific sequence. It is submitted that the Examiner is in error in this rejection, especially in view of recent Federal Circuit precedent.

Applicants note that claim 22 has been amended to specify that the RASSF1 gene is the human RASSF1 gene, thus limiting the scope of the RASSF1 gene. The human RASSF1 gene was known to skilled artisans, as acknowledged by the Examiner's citation of the Dammann et al. reference. The state of scientific knowledge is to be considered in determining compliance with the written description requirement. *Capon v. Eshhar*, 76 U.S.P.Q.2d 1078, 1084 (Fed. Cir. 2005). The "written description requirement may be satisfied 'if in the knowledge of the art the disclosed function is sufficiently correlated to a particular, known structure.'" *Id.*, citing *Amgen, Inc. v. Hoechst Marion Roussel, Inc.*, 65 U.S.P.Q.2d 1385, 1398 (Fed. Cir. 2003). *See also, Enzo Biochem, Inc. v. Gen-Probe, Inc.*, 63 U.S.P.Q.2d 1609, 1613 (Fed. Cir. 2002) (clarifying that Eli Lilly did not hold that all functional descriptions of genetic material necessarily fail as a matter of law to meet the written description requirement; rather, the requirement may be satisfied if in the knowledge of the art the disclosed function is sufficiently correlated to a particular, known structure). The court concluded in *Capon* that "when the prior art includes the nucleotide information, precedent does not

set a *per se* rule that the information must be determined afresh.” *Capon*, 76 U.S.P.Q.2d at 1084-1085. Because the prior art includes the nucleotide information of the human RASSF1 gene, it is submitted that claim 22 as amended complies with the written description requirement in accordance with the principles enunciated in *Capon*.

In view of the amendments to the claims and the above remarks, it is submitted that claim 22 satisfies the written description requirement of 35 U.S.C. § 112, first paragraph. Withdrawal of this rejection is requested.

Enablement Rejection

The Examiner has rejected claims 1, 2, 4, 6-8, 11-17 and 19-25 under 35 U.S.C. § 112, first paragraph for lack of enablement. The Examiner notes that the specification is enabling for *in vitro* directed methylation. However, she contends that this disclosure does not enable *in vivo* directed methylation. To support her rejection, the Examiner cites Mathieu et al. (*J Cell Sci* 117:4881-4888, 2004) and Caplen et al. (*Gene* 252:95-105, 2000). Mathieu et al. is a post-filing publication that the Examiner cites for the proposition that there is a lack of understanding with respect to the exact mechanism of RNA directed DNA methylation. The Examiner acknowledges that the introduction of dsRNA targeted to a specific gene results in an attenuation of the expression of the targeted gene via RNAi, she contends that the degree of attenuation and the length of time that attenuation is achieved is not predictable. The Examiner then concludes that since the effects of RNAi are unpredictable *in vivo*, the effects of the correlated DNA methylation are considered unpredictable as well. Caplen et al. is a pre-filing publication that the Examiner cites to demonstrate the unpredictability between *in vitro* cell lines and the effects of RNAi, especially with respect to vertebrate cells. It is submitted that the Examiner is in error in this rejection.

First, Applicants note that Caplen et al. was published more than three years prior to the filing of the present application. There can be no dispute that the state of the art has advanced significantly between Caplen et al.’s publication date and the present application’s filing date. The advancements in the art clearly demonstrate that a skilled artisan would expect that the present

invention would function *in vivo*, and hence are fully enabled. In fact, the very authors of Caplen et al., have shown subsequently to the Caplen et al. publication date and prior to the filing date of the present application that sequence-specific dsRNA mediated interference of gene expression has been observed in mammalian cells. *See*, Caplen et al. (*Hum Mol Genet* 11:175-184, 2002, copy attached) at page 180, last paragraph right column. The authors note that the system reported in this paper was a transient co-transfection system, but that they also have evidence that the same small dsRNA methodology can be used to inhibit endogenous gene expression. *Id. See also*, Caplen et al. (*Proc Natl Acad Sci USA* 98:9742-9747, 2001, reference AH in Information Disclosure Statement) which describes the use of siRNAs for gene-specific inhibition of expression in human and mouse cell lines and Castanotto et al. (*RNA* 8:1454-1460, 2002, copy attached) which describes a sequence-specific RNAi effect in mammalian cells using siRNA or shRNA.

In addition, it has been shown that small interfering RNA (siRNA) is effective for sequence-specific suppression of heterologous and endogenous gene expression in *Xenopus* embryos. *See*, Zhou et al. (*Nucl Acids Res* 30:1664-1669, 2002, copy attached) at page 1668, last paragraph right column. Similar results have been report for mouse embryos (*see*, Calegari et al. (*Proc Natl Acad Sci USA* 99:14236-14240, 2002, copy attached) at page 14238, last paragraph right column) and for mouse (*see*, McCaffrey et al., *Nature* 418:38-39, 2002 (copy attached) and Lewis et al., *Nature Genetics* 32:107-108, 2002 (copy attached)). Finally, it has also been shown that short hairpin RNA (shRNA) is effective for inducing gene silencing in mammalian somatic cells. *See*, Paddison et al. (*Genes Develop* 16:948-958, 2002, reference AX in Information Disclosure Statement) at page 956, paragraph bridging the left and right columns. Thus, Applicants submit that a skilled artisan would reasonably predict that small double stranded RNA functioned in mammalian cells and in vertebrate animals, including mice, for sequence-specific suppression of gene expression. That is, a skilled artisan would predict with a reasonable expectation of success that small double stranded RNA would suppress gene expression in mammalian cells *in vitro* and *in vivo*.

Second, Applicants note that Mathieu et al. is directed to a study of RNA-directed DNA methylation in plants with a brief discussion of RNA-directed heterochromatin formation in fission

yeast. Plants differ from animals in many respects. For example, in plants Dicer is located in the nucleus. Plants have enzyme classes that are not found in animals, such as RNA dependant DNA polymerases, so an interplay between the RNA and DNA worlds is known in plants. In contrast, Dicer in mammals is located in the cytoplasm and – except for telomerase – there is no known RNA-dependant DNA polymerase in mammals. So plants and humans are quite far apart. Although methylation in mammalian cells may be controversial (for example, Svoboda et al. (*Nucl Acids Res* 32:3601-3606, 2004, copy attached) and Park et al. (*Biochem Biophys Res Comm* 323:275-280, 2004, copy attached) report that they were unsuccessful in triggering methylation in mammalian cells, whereas Kawasaki and Taira (*Nature* 431:211-217, 2004, copy attached) and Morris et al. (*Science* 305:1289-1292, 2004, copy attached) report that they were successful in triggering methylation in mammalian cells), Applicants have proved it by the studies set forth in the present application. In addition, Applicants note that new studies are showing a role for siRNAs in heterochromatin behavior. See, Agrawal et al. (*Microbiol Mol Biol Rev* 67:657-685, 2003, copy attached). Although Mathieu et al. may conclude that the mechanism for translating RNA signals into DNA methylation imprints is not known, Applicants are not required to determine and set forth the precise mechanism of DNA methylation. Applicants have shown that siRNA is capable of targeting sequences that can be methylated and methylating such sequences. Thus, Applicants submit that a skilled artisan would predict with a reasonable expectation of success that siRNA can function to direct the methylation of a target sequence on the basis of the data provided in the present application. In addition, in view of the previously described references, Applicants submit that a skilled artisan would reasonably predict that this demonstrated methylation would be effective *in vivo* as well as *in vitro*.

Finally, the Examiner asserted that five variables would have to be optimized for practicing the claimed invention *in vivo* and that each of these variables would have to be determined empirically. As a result the Examiner contends that it would require undue trial and error experimentation to practice the invention because the art of attenuating gene expression by introducing dsRNA into a cell or organism is neither routine nor predictable. Other than citing

Caplen et al. and Mathieu et al., the Examiner has not provided any basis for supporting the contention that the art of attenuating gene expression is neither routine nor predictable. As shown in the above discussion concerning these cited references, Applicants have provided strong scientific evidence that the art is reasonably predictable with respect to *in vitro* and *in vivo* effects.

The Examiner has admitted that the introduction of dsRNA targeted to a specific gene may result in the attenuation of expression of the targeted gene. Although the degree and length of time of attenuation may not be predictable, they are readily determined by using techniques well known in the art. While the determination of this attenuation may require some experimentation, it is submitted that such experimentation is not undue, nor has the Examiner provided any evidence to establish otherwise. Similarly, techniques are well known in the art, including the art that Applicants cited above, for (1) determining the siRNA to utilize, (2) determining the form of the oligonucleotide, (3) determining the mode of delivery, (4) determining the amount of oligonucleotide to be delivered and (5) determining the length of time of the viability of the oligonucleotide. Although these techniques may require some experimentation for a given target sequence, it is submitted that such experimentation is not undue, nor has the Examiner provided any evidence to establish otherwise. In addition, the Examiner has asserted that these variables would need to be optimized. Optimization is not the test for enablement. The test is whether a skilled artisan can practice the invention on the basis of the information provided in the application in view of the information known in the art. Applicants submit that a skilled artisan can practice the invention described in the present application and that it is not necessary to optimize the variables noted by the Examiner prior to practicing the invention. Applicants further submit that the Examiner has not provided any evidence that the claimed invention cannot be practiced on the basis of the description in the application in view of the knowledge in the art.

It is well established that the enablement requirement is satisfied if, given what they already know, the specification teaches those in the art enough that they can make and use the invention without “undue experimentation.” *Genentech, Inc. v. Novo Nordisk, A/S*, 42 U.S.P.Q.2d 1001, 1004 (Fed. Cir. 1997); *In re Vaeck*, 20 U.S.P.Q.2d 1438, 1444 (Fed. Cir. 1991). The proper legal test is

not whether *any* experimentation is necessary, but rather whether *undue* experimentation is necessary. *See, Atlas Powder Co. v. E.I. du Pont de Nemours & Co.*, 224 U.S.P.Q. 409, 413 (Fed. Cir. 1984). Indeed, it is submitted that the articles cited herein are evidence that one of ordinary skill would be given considerable guidance by the art, and thus the Office Action fails to meet the PTO's burden of proving lack of enablement. *In re Wright*, 27 U.S.P.Q.2d 1510, 1513 (Fed. Cir. 1993); MPEP §2164.04.

In view of the above remarks, it is submitted that claims 1, 2, 4, 6-8, 11-17 and 19-25 satisfy the enablement requirement of 35 U.S.C. § 112, first paragraph. Withdrawal of this rejection is requested.

Anticipation Rejections

The Examiner has rejected claims 1, 6-8 and 25 under 35 U.S.C. § 102(b) as being anticipated by Elbashir et al. (*Nature* 411:494-498, 2001). In essence, the Examiner contends that Elbashir et al. discloses 21 bp siRNA duplexes that mediate gene inactivation via RNAi in cultured mammalian cells. Although Elbashir et al. do not disclose methylation of a target gene, the Examiner contends that the siRNAs of Elbashir et al. are necessarily considered to function by methylating a target gene because these siRNAs meet the structural limitations of the claims and the same method step is used. Thus, Examiner asserts that in order for the invention to be operative as claimed, Elbashir et al. would necessarily anticipate the claimed invention. It is submitted that the amendment of the claims obviates this rejection.

Specifically, claim 1 has been amended to specify that the target sequence is located in the promoter region of the gene that can be methylated. As taught in the present application, the siRNA is designed to be specific to this target sequence, and as a result directs the methylation of the gene. There is no disclosure or suggestion in Elbashir et al. of directing siRNA molecules to target regions that are within the promoter region of a gene to direct the methylation of the gene of interest. The amendment of claim 1 imparts structural and functional differences to the claimed siRNAs. That is, the claimed siRNAs are specific to a target sequence that is located in the promoter region of

gene that can be methylated. Because these siRNAs are not disclosed by Elbashir et al., the presently claimed invention is not anticipated by this reference.

In view of the amendments to the claims and the above remarks, it is submitted that Elbashir et al. does not anticipate the claimed invention. Withdrawal of this rejection is requested.

The Examiner has rejected claims 1, 6-8, 11-13, 18, 19 and 23-25 under 35 U.S.C. § 102(a) as being anticipated by Miyagishi et al. (*Nature Biotechnology* 19:497-500, 2002). In essence, the Examiner's reasons for this rejection are similar to those described above with respect to the rejection over Elbashir et al. In addition to a disclosure that is similar to Elbashir et al., the Examiner also notes that Miyagishi et al. discloses the use of the U6 promoter to drive expression of the siRNAs. It is submitted that the amendment of the claims obviates this rejection.

Specifically, claim 1 has been amended to specify that the target sequence is located in the promoter region of the gene that can be methylated. As taught in the present application, the siRNA is designed to be specific to this target sequence, and as a result directs the methylation of the gene. There is no disclosure or suggestion in Miyagishi et al. of directing siRNA molecules to target regions that are within the promoter region of a gene to direct the methylation of the gene of interest. The amendment of claim 1 imparts structural and functional differences to the claimed siRNAs. That is, the claimed siRNAs are specific to a target sequence that is located within the promoter region of gene that can be methylated. Because these siRNAs are not disclosed by Miyagishi et al., the presently claimed invention is not anticipated by this reference.

In view of the amendments to the claims and the above remarks, it is submitted that Miyagishi et al. does not anticipate the claimed invention. Withdrawal of this rejection is requested.

The Examiner has rejected claims 1, 6-8, 11, 17-20 and 25 under 35 U.S.C. § 102(a) or (e) as being anticipated by Fire et al. (US 6,506,559 B1). In essence, the Examiner's reasons for this rejection are similar to those described above with respect to the rejection over Elbashir et al. In addition to a disclosure that is similar to Elbashir et al., the Examiner also notes that Fire et al. discloses various target genes, promoters, targeted inhibition and expression constructs. It is submitted that the amendment of the claims obviates this rejection.

Specifically, claim 1 has been amended to specify that the target sequence is located within the promoter region of the gene that can be methylated. As taught in the present application, the siRNA is designed to be specific to this target sequence, and as a result directs the methylation of the gene. There is no disclosure or suggestion in Fire et al. of directing siRNA molecules to target regions that are within the promoter region of a gene to direct the methylation of the gene of interest. The amendment of claim 1 imparts structural and functional differences to the claimed siRNAs. That is, the claimed siRNAs are specific to a target sequence that is located in the promoter region of gene that can be methylated. Because these siRNAs are not disclosed by Fire et al., the presently claimed invention is not anticipated by this reference.

In view of the amendments to the claims and the above remarks, it is submitted that Fire et al. does not anticipate the claimed invention. Withdrawal of this rejection is requested.

The Examiner has rejected claims 1, 11-14, 18-21 and 23-25 under 35 U.S.C. § 102 (e) as being anticipated by Graham (US 6,573,099 B1). In essence, the Examiner's reasons for this rejection are similar to those described above with respect to the rejection over Elbashir et al. In addition to a disclosure that is similar to Elbashir et al., the Examiner also notes that Graham discloses various expression constructs and delivery systems including vectors and liposomes. It is submitted that the amendment of the claims obviates this rejection.

Specifically, claim 1 has been amended to specify that the target sequence is located within the promoter region of the gene that can be methylated. As taught in the present application, the siRNA is designed to be specific to this target sequence, and as a result directs the methylation of the gene. Although Graham broadly defines a gene to include transcriptional and/or translational regulatory sequences and/or coding region and/or non-translated sequences, there is no disclosure or suggestion in Graham of directing siRNA molecules to target regions that are within the promoter region of the gene of interest to direct the methylation of the gene of interest. The amendment of claim 1 imparts structural and functional differences to the claimed siRNAs. That is, the claimed siRNAs are specific to a target sequence that is located within the promoter region of gene that can

be methylated. Because these siRNAs are not disclosed by Graham, the presently claimed invention is not anticipated by this reference.

In view of the amendments to the claims and the above remarks, it is submitted that Graham does not anticipate the claimed invention. Withdrawal of this rejection is requested.

The Examiner has rejected claims 1, 6-8, 11-15, 18, 19, 21, 23 and 25 under 35 U.S.C. § 102(a) as being anticipated by Qin et al. (*Proc Natl Acad Sci USA* 100:183-188, 2003). In essence, the Examiner's reasons for this rejection are similar to those described above with respect to the rejection over Elbashir et al. In addition to a disclosure that is similar to Elbashir et al., the Examiner also notes that Qin et al. discloses lentiviral-mediated delivery and amplification. It is submitted that the amendment of the claims obviates this rejection.

Specifically, claim 1 has been amended to specify that the target sequence is located within the promoter region of the gene that can be methylated. As taught in the present application, the siRNA is designed to be specific to this target sequence, and as a result directs the methylation of the gene. There is no disclosure or suggestion in Qin et al. of directing siRNA molecules to target regions that are within the promoter region of a gene to direct the methylation of the gene of interest. The amendment of claim 1 imparts structural and functional differences to the claimed siRNAs. That is, the claimed siRNAs are specific to a target sequence that is located within the promoter region of gene that can be methylated. Because these siRNAs are not disclosed by Qin et al., the presently claimed invention is not anticipated by this reference.

In view of the amendments to the claims and the above remarks, it is submitted that Qin et al. does not anticipate the claimed invention. Withdrawal of this rejection is requested.

Obviousness Rejection

The Examiner has rejected claims 1, 2, 4, 6-8, 22 and 25 under 35 U.S.C. § 103(a) as being obvious over Elbashir et al. in view of Mette et al. (*The EMBO J* 19:5194-5201, 2000) further in view of Dammann et al. (*Nature Genetics* 25:315-319, 2000). Elbashir et al. is cited for the same disclosure as above. Mette et al. is cited for its disclosure that transcriptional gene silencing (TGS)

accompanied by de novo methylation of a target promoter can be triggered by dsRNA containing promoter sequences in plants. Mette et al. is further cited for its disclosure of a correlation between post-transcriptional gene silencing (PTGS) in plants with RNAi in other organisms. Dammann et al. is cited for its disclosure of the RASSF1A transcript, as well as methylation of the CpG -island promoter region is correlated with loss of expression. On the basis of these teachings, the Examiner contends that it would have been obvious to design a siRNA as taught by Elbashir et al. to methylate a gene of interest as taught by Mette et al., in which the gene of interest is RASSF1A taught by Dammann et al. with the motivation of studying RASSF1 gene role in lung tumors with a reasonable expectation of success. It is submitted that (a) the amendment of the claims obviates this rejection and (b) there is no suggestion in the cited prior art that would lead to the present invention.

First, claim 1 has been amended to specify that the target sequence is located in the promoter region of the gene that can be methylated. As taught in the present application, the siRNA is designed to be specific to this target sequence, and as a result directs the methylation of the gene. There is no disclosure or suggestion in Elbashir et al. of directing siRNA molecules to target regions that are within the promoter region of a gene to direct the methylation of the gene of interest. The amendment of claim 1 imparts structural and functional differences to the claimed siRNAs. That is, the claimed siRNAs are specific to a target sequence that is located in the promoter region of gene that can be methylated. Because these siRNAs are not disclosed by Elbashir et al., Elbashir et al. does disclose this element of the claimed invention.

Second, this element is not provided by either of the other cited references. It is clear that this element is not disclosed in Dammann et al. which merely discloses the RASSF1A transcript and loss of expression resulting from methylation of the promoter. There is no disclosure of siRNA molecules in Dammann et al. that are specific to a target sequence of a gene that is located in the promoter region of a gene that can be methylated. It is also submitted that the element lacking from Elbashir et al. is not provided by Mette et al.

Specifically, although Mette et al. teaches that dsRNA induces a post-transcriptional gene silencing (PTGS) process that is similar to RNAi in diverse organisms, the promoter methylation

described in Mette et al. is not a PTGS process but is a transcriptional gene silencing (TGS) process. TGS and PTGS are distinct pathways in plants, although there has been some blurring in the identity between these two pathways. See, Agrawal et al. (*Microbiol Mol Biol Rev* **67**:657-685, 2003, copy attached). This TGS process is only illustrated in plants. There is no disclosure in Mette et al. that this TGS process is similar to any process in other organisms. In addition, it is submitted that TGS is different from PTGS, and thus the fact that similar processes or gene products may be used for PTGS in plants and other organisms is not relevant. Specifically, it is submitted that TGS should not be considered to be the same as PTGS, especially in view of the discussion in Mette et al. at page 5199, starting with the penultimate paragraph in the left column. Mette et al. suggests that the disparity that they observed in their present experiments with other cases of PTGS in plants could “reflect differences between RNA-mediated TGS and PTGS, which take place in separate cellular compartments that could present distinct environments for RNA-RNA interactions.” See page 5199, top right column. This fact was also demonstrated in Mette et al.’s failure to produce *trans*-acting dsRNA as noted on page 5199, top right column.

Furthermore, Mette et al. is directed to plants which differ from animals. In plants, Dicer is located in the nucleus. Plants have enzyme classes that are not found in animals, such as RNA dependant DNA polymerases, so an interplay between the RNA and DNA worlds is known in plants. In contrast, Dicer in mammals is located in the cytoplasm and – except for telomerase – there is no known RNA-dependant DNA polymerase in mammals. So plants and humans are quite far apart. Although methylation in mammalian cells may be controversial (for example, Svoboda et al. (*Nucl Acids Res* **32**:3601-3606, 2004, copy attached) and Park et al. (*Biochem Biophys Res Comm* **323**:275-280, 2004, copy attached) report that they were unsuccessful in triggering methylation in mammalian cells, whereas Kawasaki and Taira (*Nature* **431**:211-217, 2004, copy attached) and Morris et al. (*Science* **305**:1289-1292, 2004, copy attached) report that they were successful in triggering methylation in mammalian cells), Applicants have proved it by the studies set forth in the present application. In addition, Applicants note that new studies are showing a role for siRNAs in heterochomatin behavior. See, Agrawal et al. (*Microbiol Mol Biol Rev* **67**:657-685, 2003, copy

attached). Thus, in view of these facts, it is also submitted that the element lacking from Elbashir et al., i.e., siRNAs that are specific to a target sequence that is located within the promoter region of gene that can be methylated in mammals, is not provided by Mette et al.


In view of the amendments to the claims and the above remarks, it is submitted that Elbashir et al. in view of Mette et al. and further in view of Dammann et al. does not render the claimed invention obvious. Withdrawal of this rejection is requested.

Concluding Remarks

In view of the above remarks, it is submitted that the claims satisfy the requirements of the patent statutes and are patentable over the prior art of record. Reconsideration of this application and early notice of allowance is requested. The Examiner is invited to telephone the undersigned if it will assist in expediting the prosecution and allowance of the instant application.

Respectfully submitted,

ROTHWELL, FIGG, ERNST & MANBECK, p.c.

By  _____
Jeffrey L. Ihnen
Registration No. 28,957
Attorney for Applicants
1425 K Street, N.W., Suite 800
Washington, D.C. 20005
Phone: 202-783-6040
Fax: 202-783-6031

Attachments: Caplen, N.J. et al. (2002). Rescue of polyglutamine-mediated cytotoxicity by double-stranded RNA-mediated RNA interference. *Hum Mol Genet* 11:175-184.

Castanotto, D. et al. (2002). Functional siRNA expression from transfected PCR products. *RNA* 8:1454-1460.

Zhou, Y. et al. (2002). Post-transcription suppression of gene expression in *Xenopus* embryos by small interfering RNA. *Nucl Acids Res* 30:1664-1669.

- Calegari, F. et al. (2002). Tissue-specific RNA interference in postimplantation mouse embryos with endoribonuclease-prepared short interfering RNA. *Proc Natl Acad Sci USA* **99**:14236-14240
- McCaffrey, A.P. et al. (2002). RNA interference in adult mice. *Nature* **418**:38-39.
- Lewis, D.L. et al. (2002). Efficient delivery of siRNA for inhibition of gene expression in postnatal mice. *Nature Genetics* **32**:107-108.
- Svoboda, P. et al. (2004). Lack of homologous sequence-specific DNA methylation in response to stable dsRNA expression in mouse oocytes. *Nucl Acids Res* **32**:3601-3606.
- Park, C.W. et al. (2004). Double-stranded siRNA targeted to the huntingtin gene does not induce methylation. *Biochem Biophys Res Comm* **323**:275-280.
- Kawasaki, H. and Taira, K (2004). Induction of DNA methylation and gene silencing by short interfering RNAs in human cells. *Nature* **431**:211-217.
- Morris, K.V. et al. (2004). Small interfering RNA-induced transcriptional gene silencing in human cells. *Science* **305**:1289-1292.
- Agrawal, N. et al. (2003). RNA interference: biology, mechanism, and applications. *Microbiol Mol Biol Rev* **67**:657-685.